RESULTS

RESULTS OF THE TEST TO EVALUATE THE ACUTE TOXICITY FOLLOWING A SINGLE ORAL ADMINISTRATION IN THE RAT

TEST ARTICLE : Padina pavonica

STUDY No : 072335 - D01

EXPERIMENTAL DESIGN :

ADMINISTRATION				ANIMALS		
Date	Dose level mg/kg	Volume ml/kg	Conc. %	Sex	Mean Weight g	Number
21/10/97	2000	20	10	Male	163	10
				Female	135	10

MORTALITY :

Dose level mg/kg	Sex	CUMULATIVE MORTALITY Day 1 Hours 1/4	1	2	4	Day 2	3	4	5	6	7	8	9	10	11	12	13	14	15	TOTAL MORTALITY %
2000	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

CLINICAL SIGNS

TEST ARTICLE : Padina pavonica

PROTOCOL N° : 944/007-D
STUDY N° : 072335 - D01

Group 1 (2000 mg/kg)

Male nos. 01101-01102-01103-01104-01105-01106-01107-01108-01109-01110
No abnormal clinical sign was observed.

Female nos. 01201-01202-01203-01204-01205-01206-01207-01208-01209-01210
No abnormal clinical sign was observed

EVOLUTION OF THE MALE BODY WEIGHTS (in grammes)

TEST ARTICLE : Padina pavonica

STUDY No : 072335 - D01

	D-1	D1	D8	D15	D15 - D-1	Dead
Group 1 (2000 mg/kg)						
No 01101	194	172	258	313	119	
No 01102	184	161	234	279	95	
No 01103	179	159	226	279	100	
No 01104	182	160	234	298	116	
No 01105	180	158	231	279	99	
No 01106	181	160	226	278	97	
No 01107	184	161	236	293	109	
No 01108	191	168	240	295	104	
No 01109	183	159	228	275	92	
No 01110	190	167	240	290	100	
MEAN	184.80	162.50	235.30	287.90	103.10	
S.D.	5.09	4.74	9.48	12.09	8.93	
C.V. (%)	2.76	2.92	4.03	4.20	8.66	

EVOLUTION OF THE FEMALE BODY WEIGHTS (in grammes)

TEST ARTICLE : Padina pavonica

STUDY No : 072335 - D01

	D-1	D1	D8	D15	D15 - D-1	Dead
Group 1 (2000 mg/kg)						
No 01201	157	133	191	218	61	
No 01202	148	131	169	189	41	
No 01203	149	128	170	181	32	
No 01204	154	135	188	218	64	
No 01205	155	138	179	197	42	
No 01206	157	137	188	213	56	
No 01207	151	134	172	186	35	
No 01208	155	139	173	185	30	
No 01209	152	135	178	201	49	
No 01210	152	135	179	197	45	
MEAN	153.00	134.50	178.70	198.50	45.50	
S.D.	3.13	3.27	7.97	13.81	11.88	
C.V. (%)	2.04	2.43	4.46	6.96	26.11	

NECROPSY OBSERVATIONS

TEST ARTICLE : Padina pavonica

STUDY N° : 072335 - D01

Group 1 (2000 mg/kg)

* Animals euthanatized on study termination (D15) :

Male nos. 01101-01102-01103-01104-01105-01106-01107-01108-01109-01110

No macroscopically detectable abnormality was noted.

Female nos. 01201-01202-01203-01204-01205-01206-01207-01208-01209-01210

No macroscopically detectable abnormality was noted.

APPENDIX

Chrysalis
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Protocol N° 944/007-D of 15 October 1997

Study Padina pavonica - Innocuity study following a single oral administration in the rat

Study Sponsor :
Laboratoires LAPHAL
B.P. 7
13718 ALLAUCH CEDEX
FRANCE

- **TEST ARTICLE** Padina pavonica

- **AIM OF THE STUDY** to determine the innocuity of the test article following one single oral (gavage) administration in the rat

- **STUDY PERFORMED ACCORDING TO THE STANDARD PROTOCOL N° INNOC 10/97 HERE ATTACHED**

- **STUDY SPONSOR**

  - Address . Laboratoires LAPHAL
    B P 7
    13718 ALLAUCH CEDEX
    FRANCE

  - Study Monitor Mme C SALES

- **TESTING FACILITY**

  - Address . CHRYSALIS
    Preclinical Services - Europe
    Les Oncins - BP 0118
    69593 L'ARBRESLE CEDEX
    FRANCE

  - Study Director C RUAT, D U.T Biologie Appliquée, Diplôme E P H E.

- **TEST ARTICLE**

  - Appearance to be defined in the report

  - Purity assumed to be 100 % unless otherwise advised by the Sponsor

  - Storage at room temperature unless otherwise advised by the Sponsor Other storage conditions should be specified by the Sponsor

    The Study Sponsor is responsible for sending a certificate of conformity to the Study Director for each batch of test or control article supplied to Chrysalis Preclinical Services - Europe.
    This certificate documents that appropriate checking procedures have been used to ensure that the test or control article conforms to established specifications and is that intended for use in the study.

- **VEHICLE AND CONTROL ARTICLE** (if appropriate)

  Will be decided by the Study Director (water, pure olive oil, 1 % carboxymethylcellulose, or any other appropriate vehicle), unless otherwise specified by the Sponsor The vehicle used will be indicated in the report

- **FORMULATION OF THE TEST ARTICLE** (if appropriate)

  - Preparation the test article will be prepared as a suspension or a solution in the vehicle
  - Storage at room temperature
  - Stability of the test article in the vehicle information to be supplied by the Study Sponsor
  - Frequency of preparation once only before the treatment The formulation will be used within 4 hours of preparation and assumed to be stable, unless otherwise specified by the Sponsor

    or :
  - Test article used as supplied

- **OTHER INFORMATION**

  - Guidelines
    - OECD Guideline 401 (1987)
    - EEC Directives 92/69 (1992)
  - No draft report will be supplied.
  - GLP · OECD

- **SCHEDULE OF THE STUDY**

  - Start of treatment within 3 weeks of the receipt of the signed protocol
  - Despatch of the report within 12 weeks of the start of treatment

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	Signature	Date
Issued by the Study Director		15 October 1997
Accepted by the Study Sponsor		16 octobre 1997

Standard protocol N° INNOC 10/97

Study Innocuity study following a single oral administration in the rat

Testing facility CHRYSALIS
Preclinical Services - Europe
Les Oncins - B P. 0118
69593 L'ARBRESLE CEDEX
FRANCE

EXPERIMENTAL PROCEDURE

STUDY · INNOCUITY STUDY FOLLOWING A SINGLE ORAL ADMINISTRATION IN THE RAT

REGULATIONS . Adapted from
- OECD Guideline 401 (1987)
- EEC Directives 92/69 (1992)

THE CONTENT OF THIS PROTOCOL REPRESENTS OUR INTERPRETATION OF THE STUDY OBJECTIVES AND THE REQUIREMENTS OF THE REGULATORY GUIDELINES

ALL PROCEDURES DESCRIBED IN THIS PROTOCOL ARE THE SUBJECT OF DETAILED DEPARTMENTAL STANDARD OPERATING PROCEDURES

1. AIM OF THE STUDY

To determine the innocuity of the test article in the rat following one single oral (gavage) administration.

2. TEST SYSTEM AND ENVIRONMENT

2.1. SPECIES, STRAIN, SUPPLIER AND SPECIFICATIONS

- Species, strain Rat Ico OFA.SD (IOPS Caw)
- Supplier IFFA-CREDO (B.P. 0109, 69592 L'Arbresle Cedex - France)
- Justification one of the rodent species acceptable to regulatory authorities. Background data for the strain available at the testing facility No known contra-indication to its use
- Number of animals in the study . 20 (10 males, 10 females)
- Age at initiation of treatment 5 to 7 weeks old
- Body weight range at initiation of treatment
  - males 130 to 220 g
  - females 120 to 190 g

2.2. ENVIRONMENT AND HUSBANDRY

- Housing due to the small number of animals, this study may be housed in the same room as other animals of the same species, in an air-conditioned building (building K, barrier protected unit)
  - temperature 20 to 24°C (target values),
  - relative humidity 40 to 70 % R H (target values),
  - air changes minimum 15 air changes per hour,
  - lighting cycle 12 hours light (artificial)/12 hours dark

- Caging animals housed in groups of up to 5 of the same sex and dose group in polycarbonate cages type MI (365 x 225 x 180 mm)

- Bedding dust-free sawdust made from spruce tree wood, analysed at least twice a year for chemical and bacterial contaminants A copy of certificates of analysis is kept at the testing facility

2.3. DIET AND WATER

- Diet pelleted complete diet, *ad libitum* (Diet reference A04 C10, Usine d'Alimentation Rationnelle, Villemoisson, 91360 Epinay s/Orge, France), sterilised by irradiation and analysed for the absence of chemical and bacteriological contaminants. Animals will be fasted overnight (15 to 20 hours) before dosing. They will be given food 3 to 4 hours after dosing.

- Water softened and filtered mains drinking water, *ad libitum* analysed at least once a year for chemical contaminants and at least twice a year for bacterial contaminants (Laboratoire de Chimie de l'environnement du Département d'Ecologie Urbaine de la ville de Lyon).

- Contaminants no contaminants are known to be present in the diet or water at levels which might interfere with achieving the objective of the study
  Certificates of analysis for the diet and for the water will be maintained in the archives of the testing facility

3. PRE-TREATMENT PROCEDURES

- Animal health procedure clinical inspection for ill-health on arrival and then on the day before dosing

- Acclimatisation period 5 days minimum between animal arrival and start of treatment

- The range of weight variation in the animals used should not exceed for each sex and group ± 20 % of the mean weight

- Identification of the animals ear notches

- Identification of the cages colour coded label with test number, group number, sex and animal numbers, code number of the test article, the starting date of the test.

4. TREATMENT

4.1. EXPERIMENTAL DESIGN

Group number	Group designation	Dose level (mg/kg)	Number of animals Males	Females
1	Limit dose	2000*	10	10

* if applicable

4.2. ROUTE AND METHOD OF ADMINISTRATION

- Route oral

- Justification of the route possible route of exposure in man , the oral route is also a reference route for maximal exposure

- Method single oral administration by gastric gavage using a plastic cannula

- Volume administered
  - will not exceed 20 ml/kg for aqueous preparations,
  - will not exceed 10 ml/kg for other vehicles

  Individual dose volumes will be calculated using the body weight on the day of dosing.

4.3. FREQUENCY AND DURATION OF TREATMENT

- Frequency one single administration (day 1)
- Duration . after dosing, the animals will be maintained for a 14 day observation period

Surviving animals will be killed on day 15

5. EXAMINATIONS PERFORMED

5.1. MORBIDITY/MORTALITY

Animals observed 15 minutes after administration of the test article, then at 1, 2 and 4 hours, and daily for the study period
Animals found dead during the main study will be submitted to necropsy

5.2. CLINICAL SIGNS

Animals observed 15 minutes after administration of the test article, then at 1, 2 and 4 hours, and daily for the 14 day study period The nature of the clinical signs will be recorded.

After the 14 day observation period, if abnormal clinical signs persist, these examinations will be continued with agreement of the Sponsor (at extra cost)

The animals will be weighed on the day before treatment (day - 1), immediately before treatment (day 1), on days 8 and 15 and at death from day 2 onwards

5.3. PATHOLOGY

5.3.1. NECROPSY

- A necropsy will be performed on all animals dying during the study At the end of the 14 day study period and after the final observation (day 15), all surviving animals will be euthanatized by carbon dioxide inhalation and necropsied.

- All animals (including found dead/moribund animals) will be submitted to full necropsy procedure including an examination of
  - the external surface,
  - all orifices,
  - the thoracic, abdominal and pelvic cavities and viscera.

5.3.2. ORGAN/TISSUE PRESERVATION AND HISTOPATHOLOGY (at extra cost)

For all the animals surviving for 24 hours or more after administration of the test article, organs with macroscopic lesions will be sampled and kept in fixative (10 % formalin). With the agreement of the Sponsor, sections will be stained with Hematoxylin and Eosin (H. and E) and examined by a pathologist (at extra cost)

6. DATA EVALUATION

Historical data from control rats will be used to assess effects Analysis of body weights gain will be performed.

Mortality rate will be calculated as a percentage to determine the innocuity or degree of toxicity of the test article.

7. QUALITY ASSURANCE

This study will be subjected to Quality Assurance procedures in compliance with "O E.C D Principles of Good Laboratory Practice" concerning Mutual Acceptance of Data in the Assessment of Chemicals dated 12 May 1981 (C (81) 30 Final) , "Good Laboratory Practice" described in the U S. Federal Register (Food and Drug Administration) dated 22 December 1978 with subsequent revisions, of which the last is dated 15 July 1991

The standard protocol will be audited. Procedures similar to those used on this type of study are inspected periodically in the laboratory and animal areas, and data are audited periodically from a study of this type The report will be reviewed to assure that it accurately describes the methods and procedures, and that the results accurately reflect the raw data. Reports on these activities will be made to the Study Director and to Management Any analyses performed by the Study Sponsor will not be audited by the Quality Assurance Unit of Chrysalis Preclinical Services - Europe

8. REPORT

Incidental reports

The Study Sponsor will be informed promptly of any significant findings at any time during the study

Final report

The final report will be issued and 3 copies (2 bound and one unbound) in English sent to the Study Sponsor

9. ARCHIVES

The following materials will be maintained in the archives of the testing facility for the periods indicated.

Description of materials	Duration
Original protocol (and amendments if applicable), raw data and final report	5 years
Blocks and histology slides (if applicable)	5 years
Wet tissues (if applicable)	5 years
Test article	2 months

Duration of archiving starts after dispatch of the final report

Once the period of archiving is over, the materials will be destroyed, unless otherwise requested by the Sponsor